Geographic Distribution and Breeding Site Preference of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) in Cameroon, Central Africa

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ABSTRACT Presence in Cameroon of the recently introduced *Aedes* (*Stegomyia*) *albopictus* (Skuse) in association with the indigenous *Aedes aegypti* (L.) raises public heath concerns because it might alter the risk of arbovirus transmission. The breeding site and distribution of the two *Stegomyia* species are updated and reported following entomological surveys carried out in 22 localities throughout Cameroon, with a total of 1,353 containers with water visited. *Ae. aegypti* was found in every location sampled, showing higher infestation rates in northern Cameroon. Breeding populations of *Ae. albopictus* were observed in all 19 southern localities, up to the Adamaoua mountains, but the species was not recorded further north. In the area where both species are present, they were often sampled in the same larval developmental sites, suggesting convergent habitat segregation. The most frequently encountered artificial and natural breeding sites were used tires, discarded tins and plastic containers, abandoned car parts, brick holes, dead leaves on the ground, tree holes, and rock pools. Further monitoring of the demographic as well as geographic expansion of *Ae. albopictus* in this Afrotropical environment and its relationships with indigenous *Ae. aegypti* should provide insight into the biology of this highly invasive species and help to implement arboviruses surveillance programs in the area.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, larval ecology, Cameroon, Africa

Aedes (*Stegomyia*) *aegypti* (L.) and *Aedes* (*Stegomyia*) *albopictus* (Skuse) are known or potential vectors to humans of several arboviruses (Christophers 1960, Shroyer 1986, Hawley 1988). *Ae. aegypti* has a wide distribution range, being present almost worldwide, between latitudes of 45° N and 35° S. It is arguably recognized as the major vector of yellow fever and dengue viruses. *Ae. albopictus* is originally endemic to the Oriental Region where it is a proven vector of filarial worms and dengue (Hawley 1988). This highly invasive species has recently experienced rapid worldwide range expansion (Hawley 1988, Lounibos 2002, Toto et al. 2003). Increasing intercontinental trade and especially shipments of used tires have been implicated as the primary dispersal mechanism of this species (Reiter and Darsie 1984, Hawley et al. 1987, Rodhain 1996, Reiter 1998). It is now established in numerous countries throughout the world in the Americas, Europe, Africa, and Oceania.

Both species are container-breeding mosquitoes that are closely associated to humans and highly anthropophilic. *Ae. aegypti* tends to predominate in densely populated urban areas and is commonly found indoors, breeding in artificial containers used for water storage and any kind of neglected cups or jugs containing fresh water (Christophers 1960). *Ae albopictus* typically prefers suburban and rural areas, where it breeds in natural container such as tree holes, leaf axillas or bamboo internodes, and artificial containers such as discarded tin cans and tires (Hawley 1988). However, in regions where both species cohabit, their larvae are often found together in the same larval developmental site (Braks et al. 2003).

Ae. aegypti is known in Cameroon for a long time (Rageau and Adam 1952, 1953) and was probably involved in the recent yellow fever outbreaks that occurred in the northern Cameroon in 1990 and 1995 (Vicens et al. 1993, Bouchite et al. 1995). It is widespread throughout the country, although usually more abundant in the north than in the south and breeds mainly outside human dwellings, in rain-filled or water storage containers (Rickenbach and Button 1977). Breeding populations of *Ae. albopictus* were first re-

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Table 1. Distribution and prevalence of larval developmental sites for *Ae. albopictus* and *Ae. aegypti* at sites in Cameroon (March–August 2002)

No.	Localities	Geographic coordinates	Sampling date	No. inspected		No. positive (% inspected)			
						Ae. aegypti		*Ae. albopictus*	
				Containers	Premises	Containers	Premises	Containers	Premises
1	Pitoa	09° 23′ N; 13° 30′ E	Aug. 2002	25	15	19 (76.0)	12 (80.0)	0 (0.0)	0 (0.0)
2	Garoua	09° 18′ N; 13° 25′ E	Aug. 2002	52	15	41 (78.8)	7 (46.7)	0 (0.0)	0 (0.0)
3	Ngaoundéré	07° 19′ N; 13° 35′ E	Aug. 2002	74	16	49 (66.2)	11 (68.8)	0 (0.0)	0 (0.0)
4	Tibati	06° 28′ N; 12° 37′ E	Aug. 2002	38	ND	20 (52.6)	ND	4 (10.5)	ND
5	Bankim	06° 00′ N; 11° 40′ E	Aug. 2002	27	ND	12 (44.4)	ND	2 (7.4)	ND
6	Bamenda	05° 57′ N; 10° 25′ E	Mar. 2002	145	33	11 (7.6)	4 (12.1)	6 (4.1)	3 (9.1)
7	Garoua Boulai	05° 55′ N; 14° 34′ E	June 2002	51	15	23 (45.1)	7 (46.7)	11 (21.6)	3 (20.0)
8	Bafoussam	05° 27′ N; 10° 45′ E	Mar. 2002	82	18	11 (13.4)	5 (27.8)	4 (4.9)	2 (11.1)
9	Dschang	05° 23′ N; 10° 10′ E	Mar. 2002	60	30	7 (11.7)	6 (20.0)	11 (18.3)	7 (23.3)
10	Santchou	05° 17′ N; 09° 58′ E	Mar. 2002	42	25	6 (14.3)	4 (16.0)	11 (26.2)	5 (20.0)
11	Bertoua	04° 42′ N; 13° 47′ E	June 2002	66	31	15 (22.7)	10 (32.3)	28 (42.2)	16 (51.6)
12	Mbanga	04° 30′ N; 09° 32′ E	May 2002	38	14	7 (18.4)	3 (21.4)	11 (28.9)	6 (42.9)
13	Batouri	04° 21′ N; 14° 18′ E	June 2002	73	31	15 (20.5)	12 (38.7)	15 (20.5)	14 (45.2)
14	Buéa	04° 08′ N; 09° 30′ E	May 2002	57	20	19 (33.3)	12 (60.0)	14 (24.6)	10 (50.0)
15	Tiko	04° 05′ N; 09° 21′ E	May 2002	38	ND	17 (44.7)	ND	11 (28.9)	ND
16	Limbé	04° 01′ N; 09° 13′ E	May 2002	80	26	31 (38.8)	10 (38.5)	18 (22.5)	12 (46.2)
17	Douala	04° 00′ N; 09° 43′ E	May 2002	81	42	30 (37.0)	11 (26.2)	45 (55.6)	12 (28.6)
18	Ayos	03° 52′ N; 12° 37′ E	June 2002	63	23	13 (20.6)	9 (39.1)	12 (19.0)	9 (39.1)
19	Yaoundé	03° 50′ N; 11° 30′ E	June 2002	74	ND	20 (27.0)	ND	27 (36.5)	ND
20	Mbalmayo	03° 30′ N; 11° 30′ E	July 2002	84	32	23 (27.4)	14 (43.8)	29 (34.5)	17 (53.1)
21	Sangmélima	02° 55′ N; 11° 57′ E	July 2002	70	30	27 (38.6)	20 (66.7)	15 (21.4)	10 (33.3)
22	Ebolowa	02° 53′ N; 11° 10′ E	July 2002	33	36	25 (75.6)	13 (36.1)	22 (66.7)	13 (36.1)

Numbers in the left column (1–22) refer to location position on Fig. 1. ND, not determined.

corded in Cameroon in 2000 (Fontenille and Toto 2001). This species had not been observed in previous surveys, suggesting recent introduction into Cameroon.

The introduction and further thriving of *Ae. albopictus* in Cameroon, in association with indigenous *Ae. aegypti*, is of great public health concern because it alters the risk of arbovirus transmission (Gubler 2003, ProMED-mail 2003). Although dengue viruses were never isolated from Cameroon and serological tests show low specificity due to cross-reactions, evidence for high prevalence of antibodies to arboviruses, including chikungunya, dengue, West Nile, and yellow fever recently reported in febrile patients suggest putative unrecognized public health problem in such area where endemic malaria and typhoid are the primary diagnostic considerations (Ndip et al. 2004).

The current study was conducted to assess and update the distribution of *Ae. albopictus* and *Ae. aegypti* in Cameroon and to improve knowledge on their larval ecology.

Materials and Methods

Study Area and Collection Sites. Entomological surveys were conducted between March and August 2002 in 22 locations across Cameroon (ranked according to decreasing latitude in Table 1 and Fig. 1). Because human-mediated dispersal has been shown to play a significant role in the worldwide spread of *Ae. albopictus,* we focused on cities of major economic importance, spread along the main communication networks, and trade routes throughout the country.

Cameroon shows highly diversified biotopes, ranging from subarid savannahs in the north to the humid equatorial forest in the south, with strong local heterogeneities due to huge variations in altitude (from 0 to >4,000 m above sea level on Mount Cameroon), human population densities, and large-scale land alterations due to human activities (Olivry 1986). As one travels north to south, mean annual rainfalls increases from <500 to >2,000 mm, reaching >4,000 mm on the Atlantic shore southwest of the country. The number of dry season months decreases from 9 mo in the northernmost localities to <2 mo in the southern rain forest area. Mean annual temperature ranges 23–25°C with low amplitude between annual minima and maxima in central and southern Cameroon. The coastal belt experiences somewhat higher mean annual temperatures (range 26–27°C), whereas temperatures drop below 20°C in the western highlands (e.g., 18°C in sites 6 and 9). North of Ngaoundéré (site 3), mean annual temperature typically exceeds 28°C. There is considerable yearly variation in relative humidity in northern settings, whereas humidity is much more stable in the south.

Mosquito Sampling. Because of the absence of vaccine and efficient treatment against dengue, we did not catch adult mosquitoes on volunteers. Both natural (e.g., tree holes, rock holes, and snail shells) and artificial (e.g., discarded tins, car parts, and used tires) containers filled with water that may serve as breeding sites for *Ae. aegypti* or *Ae. albopictus* were inspected for the presence of mosquito larvae or pupae. To maximize occurrence of larval development sites, collections were conducted during the rainy season.

Wherever they were observed, immature stages of mosquitoes were collected using pipettes and placed into vials labeled according to the container type, locality, and date of collection. Vials were transported

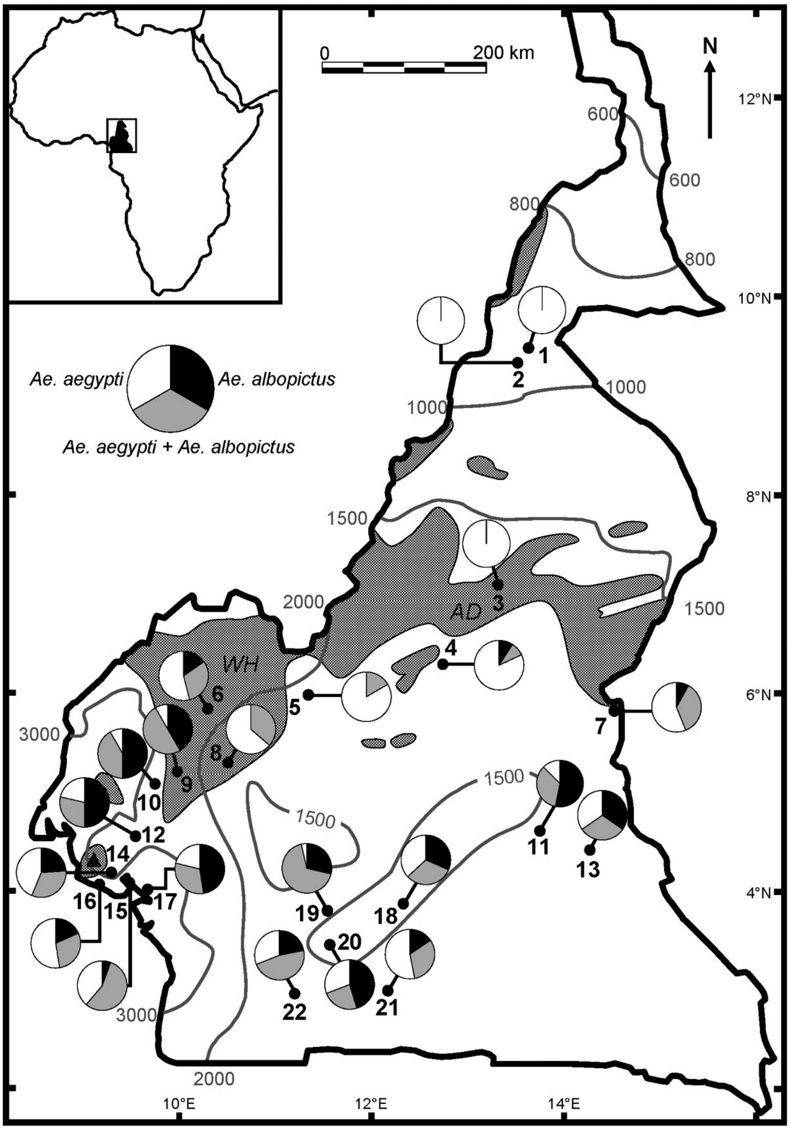

Fig. 1. Relative frequencies of breeding sites containing immature stages of *Ae. albopictus* (black), *Ae. aegypti* (white), or in which both species were found together (gray). Numbered localities are listed in Table 1. Major isohyets (in millimeters of rainfall per year) are shown as gray lines. Shaded areas are altitude >1,000 m above sea level showing Mount Cameroon (▲), western highlands (*WH*), and Adamaoua mountains (*AD*).

to the Organization of Coordination for the Fight against Endémies in Central Africa (OCEAC) entomology laboratory where larvae and pupae were grown to adults before identification. Emerging adults were identified using morphological identification keys and by reference to morphological descriptions (Edwards 1941, Hopkins 1952, Darsie 1986, Hawley 1988, Jupp 1996). A sample of male genitalia was dissected and examined under the microscope to confirm species determination. A container was recorded as positive for *Ae. albopictus* or *Ae. aegytpi* when at least one emerging adult of the species was observed.

In each of the 22 sites we visited, collections were initially conducted at a glance, outdoors, within and at the periphery of the city to include, as much as possible, different kinds of breeding sites. Then, systematic inspection of at least 14 premises was performed in a randomly selected district within the city to assess tentative premise indices (except in sites 4, 5, 15, and 19 for logistical reasons). A premise was recorded as positive when at least one breeding site contained immature stages of the *Stegomyia* species.

Results

In total, 1,353 water collections were examined from 22 locations across Cameroon (Fig. 1), among which 576 (42.6%) were colonized by immature stages of *Ae.*

Table 2. Container preferences of *Ae. albopictus* and *Ae. aegypti* in Cameroon (March–August 2002)

Container type	No. positive/no. inspected (%)		
	Ae. aegypti	*Ae. albopictus*[a]	No. shared
Used tires	124/269 (46.1)	109/237 (46.0)	60
Plastic cups	12/60 (20.0)	9/41 (22.0)	4
Buckets	48/178 (27.0)	31/178 (17.4)	15
Tanks	4/13 (30.8)	2/13 (15.4)	1
Metal cans	71/327 (16.1)	47/304 (15.5)	27
Broken bottles	10/37 (27.0)	6/35 (17.1)	3
Earthenware jars	15/20 (75.0)	3/4 (75.0)	2
Gourds	3/5 (60.0)	0/3 (0.0)	0
Car wrecks	46/104 (44.2)	32/82 (39.0)	18
Brick holes	40/74 (54.1)	28/74 (37.8)	17
Flower pots	8/8 (100.0)	0/1 (0.0)	0
Latex collection cups	19/127 (15.0)	12/127 (9.4)	10
Tree holes	11/23 (47.8)	2/14 (14.3)	0
Rock holes	4/14 (28.6)	2/10 (20.0)	1
Leaf axilla	0/5 (0.0)	1/5 (20.0)	0
Dead leaf on ground	3/27 (11.1)	4/27 (14.8)	1
Cacao shells	4/12 (33.3)	3/12 (25.0)	1
Coconut shells	0/4 (0.0)	1/4 (25.0)	0
Snail shells	0/12 (0.0)	1/11 (9.1)	0
Dead cow horns	19/34 (55.9)	3/20 (15.0)	1

[a] Container infestation rates for *Ae. albopictus* are based on data from sites 4–22, because the species was not found in the three northernmost localities.

albopictus and/or *Ae. aegypti* (Tables 1 and 2). Associated species of mosquito larvae were *Aedes vittatus* (Bigot), species from the *Aedes simpsoni* (Theobald) complex, *Anopheles gambiae* s.l. Giles, *Culex* from the group *decens* Theobald, *Culex tigripes* De Grandpré & De Charmoy, *Culex. antennatus* (Becker), *Culex quinquefasciatus* Say, *Culex perfuscus* Edwards, *Culex duttoni* Theobald, and *Eretmapodites quinquevittatus* Theobald.

Ae. aegypti was found in all locations sampled, whereas *Ae. albopictus* was apparently absent from the three northernmost localities (Fig. 1; Table 1). In the area where only *Ae. aegypti* was found (sites 1–3), it occurred in >66% of the containers surveyed. Positive breeding sites were found in 46.7–80.0% of the premises visited. Elsewhere, the frequency of occurrence of both *Stegomyia* species in water-filled containers was generally lower, except in site 22 (Table 1).

Ae. albopictus and *Ae. aegypti* colonized a variety of artificial and natural breeding places (Table 2). The volume of water in breeding sites ranged from 5 ml to 100 liters. In southern Cameroon, used tires, discarded tin cans, and plastic containers of all sorts (e.g., cups, bottles, buckets, and drums), earthenware jars, abandoned car parts, and brick holes are the most common larval habitat for both *Ae. albopictus* and *Ae. aegypti.* Both species also were found in latex collection cups in a hevea plantation in Tiko (site 15), as well as in rain-filled dead cow horns around slaughterhouses. The most common natural breeding sites were tree holes, rock holes, dead leaves on the ground, and cacao shells. Immature stages of *Ae. albopictus* also were found in a variety of natural containers where water could accumulate such as leaf axilla, snail shells, and coconut shells. In northern Cameroon (sites 1–3), used tires, earthenware jars, gourds and water storage pots are the most common breeding habitats for *Ae. aegypti.*

Both species of mosquito were frequently found together in the same larval habitat (Fig. 1; Table 2). When considering only localities where both species were recorded (i.e., excluding the three northernmost sampling sites), 48.5% (161/332) of *Ae. aegypti* breeding sites contained *Ae. albopictus* immatures. In turn, 54.4% (161/296) of water collections in which *Ae. albopictus* immatures were found, contained *Ae. aegypti* immatures. As a result, most habitat types were shared between species, although abundance of breeding sites for *Ae. albopictus* was only loosely correlated with abundance of breeding sites for *Ae. aegypti* (Pearson's correlation coefficient = 0.201; df = 17, P = 0.026 single-sided test).

Discussion

The widespread occurrence of *Ae. aegypti* we observed during our survey is reminiscent of the situation reported, some three decades ago, by Rickenbach and Button (1977) during their extensive sampling conducted between 1971 and 1974 throughout Cameroon. As these authors observed, we also found *Ae. aegypti* in every locality visited, although larval development sites tended to be more numerous and diverse in the tropical northern areas than in the south. The nature of the breeding sites was noticeably similar in both surveys. Based on such observation, and the overall low vaccine coverage, Rickenbach and Button (1977) highlighted a high risk of yellow fever transmission in the north. Unfortunately, later events proved them right, and at least two documented yellow fever outbreaks occurred in 1990 and 1995 in the area (Vicens et al. 1993, Bouchite et al. 1995). For the same reasons, our results therefore unequivocally suggest that the risk of yellow fever epidemics remains high in northern Cameroon.

Furthermore, we provide evidence that *Ae. albopictus* has established breeding populations in Cameroon, as was suggested recently (Fontenille and Toto 2001). This highly invasive mosquito species is well established in central and southern Cameroon where it occurs together with indigenous populations of *Ae. aegypti.* Both species were found in a variety of peridomestic and natural breeding sites, as commonly described (Christophers 1960, Yebakima et al. 1979, Hawley 1988, Fontenille and Toto 2001). They were often sampled together from the same larval developmental site. This suggests convergent habitat segregation for both species, as formerly observed in Singapore (Chan et al. 1971) and in Brazil and the United States (Braks et al. 2003). Used tires were one of the most abundant larval habitats for both species, and, presumably, one of the most productive as well. However, our exploratory sampling design and short-term survey prompt for further investigations. In particular, quantitative assessment of mosquito abundance and breeding site productivity was beyond the scope of this study, and seasonal fluctuations in vector abundance and/or species balance might not be revealed.

Ae. albopictus was recently introduced into Cameroon (Fontenille and Toto 2001), as it was in neighboring Nigeria (Savage et al. 1992) and Equatorial Guinea (Toto et al. 2003), and few data exist on the biology as well as the origin of African *Ae. albopictus* populations. In Madagascar, Fontenille and Rodhain (1989) showed that *Ae. albopictus* occurred in regions with 0–6 dry months a year, whereas *Ae. aegypti* can endure up to nine dry months a year. In Cameroon, the Adamaoua mountains bisect longitudinally the country, acting as a boundary between the tropical northern climate and the equatorial southern climate. Accordingly, it actually represents the northern range for *Ae. albopictus* in Cameroon. However, although the present distribution range of *Ae. albopictus* in Cameroon might reflect climatic incompatibilities, any conclusion drawn so far needs to be tentative and the dynamics of invasion of *Ae. albopictus* in Cameroon, its geographic as well as demographic expansion need to be monitored further.

As outlined by Gubler (2003), invasion and further spread of *Ae. albopictus* into areas where *Ae. aegypti* is endemic could have alternative outcomes of public health interest. High vector competence for a number of highly pathogenic viruses, including yellow fever and dengue viruses, has been demonstrated for *Ae. albopictus* under experimental conditions (Shroyer 1986, Mitchell 1995, Johnson et al. 2002), and wild-caught females were found naturally infected by several arboviruses of medical importance (Mitchell et al. 1992, Gerhardt et al. 2001, Holick et al. 2002). Moreover, its strong anthropophily combined with its ability to colonize both urban and periurban areas make *Ae. albopictus* a possible bridge vector that might increase the risk of introduction and further transmission of arboviruses. This is of obvious public health concern. Yet, importance of *Ae. albopictus* as an epidemic vector of human pathogens has hardly been demonstrated out of its area of origin nor has its presence so far modified indigenous arbovirus transmission dynamics. Hence, some authors suggest that *Ae. albopictus* invasion might have an indirect, beneficial effect on arboviruses transmission dynamics through its deleterious effect on endemic *Ae. aegypti* populations (Gubler 2003). Competitive displacement between the two species has been well documented, especially in the southeastern United States where *Ae. albopictus* invasion triggered decline in abundance and widespread disappearance of *Ae. aegypti* (Hobbs et al. 1991, O'Meara et al. 1995, Lounibos 2002). In Cameroon, only the dark form *Aedes aegypti formosus* (Walker) is found (Mattingly 1957, Service 1976), providing unique opportunity to explore putative competitive displacement and/or niche partitioning between the endemic *Ae. aegypti* populations and the invasive *Ae. albopictus* populations. Close monitoring of this recent or ongoing invasion process, in Africa, would undoubtedly generate instructive knowledge for the development and implementation of innovative vector control measures based on natural population suppression and/or replacement through the release of closely related species or populations.

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